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TITLE: Human Leukocyte Antigen (HLA) Genotype as a Contributor to Racial/Ethnic Differences in Breast Cancer: A Population-Based, Molecular Epidemiologic Study

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13. ABSTRACT (Maximum 200 Words)

Breast cancer incidence and mortality differ markedly across racial/ethnic groups, but known risk factors do not explain all of this variation. The human leukocyte antigen (HLA) component of the immune system, encoded by highly polymorphic genes that vary across racial/ethnic groups, has been suggested to be a biologically based risk factor for breast cancer and thus may explain some of its variation by race/ethnicity. Therefore, for a population-based series of white, black and Hispanic breast cancer cases and controls, we are determining: 1) HLA class I (A, B) and class II (DQ, DR) genotypes using advanced DNA methods; 2) whether HLA genotype is related to breast cancer across racial/ethnic groups; 3) whether the size of association and prevalence of associated HLA genotypes vary by race/ethnicity, and how much such differences explain racial/ethnic differences in breast cancer incidence; 4) whether HLA associations vary by indicators of prognosis, tumor characteristics, or known breast cancer risk factors. To date, we have transmitted DNA specimens for 912 subjects to the study collaborator for HLA typing. Typing has been conducted on 400 specimens, and work is ongoing. Statistical analyses will be undertaken once HLA typing is complete for all specimens.

14. SUBJECT TERMS
Human leukocyte antigen (HLA), genetic epidemiology, molecular epidemiology
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Introduction:

The incidence and mortality burdens of breast cancer differ markedly across racial/ethnic groups, particularly in post-menopausal women, but known risk factors do not explain all of this variation, or the majority of breast cancers. The human leukocyte antigen (HLA) component of the immune system, encoded by highly polymorphic genes that both vary across racial/ethnic groups and have been related to numerous diseases, has not been much examined for non-viral cancers. Therefore, to examine whether genetically determined aspects of immune function represent biologically-based risk factors for breast cancer and explain a portion of its variation by race/ethnicity, we are taking advantage of already collected DNA and epidemiologic data for a population-based series of post-menopausal white, black and Hispanic breast cancer cases and controls. With the DNA, we are using PCR-based, immobilized probe (sequence-specific oligonucleotide) typing to assign HLA genotypes to 912 incident invasive breast cancer cases and post-menopausal controls frequency-matched for age and race/ethnicity. We will assess whether HLA genotypes are associated with breast cancer overall and in each of the three racial/ethnic groups by comparing allele or haplotype distributions between cases and controls and quantifying the extent of association with odds ratios. We will determine if associations differ among the racial/ethnic groups by comparing allele-specific odds ratios and population prevalences of risk-associated alleles, and quantifying the proportion of racial/ethnic incidence differences explained by HLA using the relative attributable risk. As sample sizes permit, we will explore whether HLA associations relate to tumor characteristics, particularly stage at diagnosis, or known breast cancer risk factors. An association of HLA with racial/ethnic variation in breast cancer occurrence and progression would facilitate a clearer understanding of breast cancer etiology in the group affected and could contribute to more targeted methods of breast cancer prevention. In a clinical setting, HLA type theoretically could be helpful in assessing an individual woman's breast cancer risk profile and, if HLA proved to be linked to breast cancer stage or prognosis, for guiding therapeutic decisions.

Body:

Task 1: Develop study subject database from existing databases

a. *Apply eligibility criteria to the Bay Area Breast Cancer Study (BABCS) study database to select post-menopausal, blood-providing study subjects and extract relevant epidemiologic and specimen-tracking data.*

This task was completed. We have identified 425 cases and 487 controls who self-described as post-menopausal in an in-person interview, and for whom DNA was available.

b. *Link study patients to Greater Bay Area Cancer Registry database to obtain demographic, clinical and tumor characteristics.*

This task was completed, yielding a study database.

c. *Install linked study subject database into study tracking database, creating study identification (ID) numbers.*

This task was completed once IRB approval was received from the funding agency permitting us to work with identifiable human materials.

Task 2: Obtain DNA samples from storage at USC

a. *Transmit electronic file of study-subject BABCS tracking ID numbers to Dr. Engles' lab at USC.*

This task was completed.

b. *Request DNA for each patient be transmitted in 96-well trays to Dr. Erlich's lab, labeled only by unique specimen ID number.*

This task was completed. DNA samples, each comprising 1 microgram of DNA that had been dried down, were transmitted from Dr. Ingles' lab to Dr. Erlich's staff in 11 plates.

c. *Track DNA specimen transmission.*

This task was completed. Transmission of the DNA was tracked and its receipt was acknowledged by Dr. Erlich's staff.

Task 3: HLA-type DNA specimens

a. *Amplify class I (A,B) and class II (DQ,DR) loci.*

Amplification has occurred for the class I A loci for 400 samples.

b. *Type using immobilize probe linear arrays.*

The Roche Linear array assay for HLA used by the study collaborator, Dr. Erlich, is based on PCR amplification with biotinylated primers, hybridization to an immobilized (SSO) probe linear array, and detection of the probe reactivity pattern with streptavidin-HRP. For the class I loci (HLA-A and HLA-B) being typed first, two primer sets are used in a single PCR reaction to specifically amplify the polymorphic exon 2 (410 bp) and exon 3 (519 bp) of each locus. The strip contains two positive control lines or "ALL" probe, one for each exon to assure that both exons of each locus amplified.

c. *Scan probe reactivity patterns and convert patterns to genotype.*

Genotype assignment is done manually. The positive probe hits (indicated by blue lines on the strip) are recorded manually, and the probe reactivity pattern is interpreted by a computer algorithm to assign the sample genotype. The genotype assignments are done by Strip Scan program. Strips are scanned using a flat bed document scanner, the intensity of blue lines is given a pixel value, these values are interpreted by software, and the sample genotype is assigned based on the probe binding pattern.

d. *Record assay results on Excel spreadsheet and transmit back to NCCC.*

This task has not yet been completed. A spreadsheet will be produced and transmitted to NCCC after all typing is complete.

Task 4: Create study database (months 19)

a. *Merge study database, including interview and registry data, with HLA typing data*
b. *Link study database to Greater Bay Area Cancer Registry database to obtain most current patient vital status.*
c. *Strip database of all subject identifiers.*

These tasks have not been completed.

Task 5: Statistical analysis (months 20-23)

a. *Compare allele frequencies for HLA-A, -B, -DQ and -DR separately for each race.*
b. *Compute odds ratios and 95% confidence intervals*
c. *Compare across racial/ethnic groups HLA associations significant in any racial/ethnic group.*
d. *Examine whether relationships are confounded by other epidemiologic or tumor features.*
e. *Conduct logistic regression to predict breast cancer risk associated with the alleles of interest with control for confounders*

These tasks have not been completed.

- *Task 6: Summarize study findings for presentation and submission for publication in literature (months 23-24)*

These tasks have not been completed.

Key Research Accomplishments:

1) Develop study subject database from existing databases
2) Link study patients to Greater Bay Area Cancer Registry database to obtain demographic, clinical and tumor characteristics.
3) Install linked study subject database into study tracking database, creating study identification (ID) numbers.
4) Transmit electronic file of study-subject tracking ID numbers to Dr. Engles' lab at USC, where the DNA resides.
5) Request DNA for each patient be transmitted in 96-well trays to Dr. Erlich's lab, labeled only by unique specimen ID number.
6) Transmit DNA in 11 96-well plates.
7) Track DNA specimen transmission.
8) Begin typing process.
9) Amplify class I (A) loci for 400 samples.
10) Type using immobilize probe linear arrays.
11) Scan probe reactivity patterns and convert patterns to genotype.

Reportable Outcomes:

None yet.

Conclusions:

None yet.

References:

None yet.

Appendices:

None